cell yielded a threshold surround response. This was determined by listening to an audio monitor and viewing an oscilloscope that displayed responses summed by the computer. Next the luminance of the modulated annulus was increased by 0.7 log units. Then an unmodulated adapting annulus was placed in the receptive field and its luminance was varied until a threshold response was obtained from the modulated annulus, i.e., the surround was adapted by 0.7 log units. This procedure was repeated for the other adapting stimuli. With this method adaptive sensitivity is defined as the reciprocal of the adapting luminance (expressed in log units) required to produce a threshold response.

The curves shown in figure 1 are representative of the 10 X-cells and 10 Y-cells tested in this experiment. X-cells usually had their highest adaptive sensitivity outside of the receptive field center, and Y-cells usually had peak sensitivity in the middle of the receptive field. This finding was confirmed in an experiment in which adapting target size was varied. All of the adapting stimuli in this experiment had an outside diameter of 8.5° and variable inside diameter. Adaptive sensitivity was measured in the same manner described for the 1st experiment. Data were obtained for 13 X-cells and 14 Y-cells in the experiment. Most of the area-adaptive sensitivity curves for these cells were similar to those shown in figure 2. The major finding to be gleaned from figure 2 is the difference between X- and Y-cell adaptive sensitivity changes when adapting flux is placed in the center of the receptive field. X-cells, in general, are affected minimally, whereas Y-cells show an abrupt change in sensitivity. In figure 2, compare sensitivities to a 8.5° adapting spot and a $0.7° \times 8.5°$ adapting annulus.

The results of the 2 experiments presented here provide evidence that the surround's adaptation mechanism has a stronger representation in the center of the receptive field of Y-cells than in X-cells. The spatial distribution of the surround's signal sensitivity profile has not been determined for cat retinal ganglion cells because of the difficulty in separating center and surround signals in the central portion of the receptive field. Based on indirect evidence, however, it was suggested by Hickey, Winters and Pollack[10] and Hammond[11] that signal sensitivity was greater in the receptive field center of Y-cells than X-cells. In the rhesus monkey, signals from the surround mechanism can be separated from signals from the center mechanism by using chromatic stimuli. De Monasterio[12] assessed the spatial distribution of surround signal sensitivity in the rhesus monkey and reports that the sensitivity profile of Y-cells is unimodally distributed with peak sensitivity in the center of the receptive field. The profile for X-cells was found to be distributed bimodally with low sensitivity in the receptive field center. The results of the present study are consistent with De Monasterio's signal sensitivity data.

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Mo-V interactions during N_2- and NO_3^--metabolism in a N_2-fixing blue-green alga *Nostoc muscorum*[1]

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Summary. Molybdenum (Mo), a group 6B element (applied in the form of sodium molybdate at a concentration of 0.0177 ppm), is required for N_2- and NO_3^--mediated growth of *Nostoc muscorum*. The amount of growth of such Mo-containing culutures is significantly enhanced by the addition of 0.0125 ppm vanadium (V), an element belonging to group 5 of the periodic table (applied in the form of sodium vanadate). At concentrations above 0.0125 ppm V proves growth-inhibitory to *N. muscorum* cultured with or without Mo. Mo and/or V exert(s) no apparent stimulatory/inhibitory effect on NO_3^--mediated growth of the organism.

Mo is known to be an integral part of the nitrogenase and nitrate reductase enzyme systems responsible for N_2 fixation and NO_3^- reduction in microorganisms, including blue-green algae[3-5]. Until recently, the concept of a Mo-containing polypeptide co-factor common to different molybdoenzymes including nitrogenase and nitrate reductase has had considerable support from genetic[4,6-8] and biochemical[9,10] studies. It has, however, recently been shown in a free-living N_2-fixing bacterium *Azotobacter vinelandii* that the Mo-containing co-factor is exclusively involved in nitrate reductase activity[11], and that an iron-molybdenum (Fe-Mo)-containing co-factor is responsible for nitrogenase function[12-14]. Genetic studies by my group on the simultaneous functional replacement of Mo by its structural analogues tungsten (W)[15] or chromium (Cr)[16] for both N_2 and NO_3^- metabolism in *Nostoc muscorum* strongly supported the suggestion that the Mo-containing co-factor is the precursor of a Fe-mo-containing co-factor in the N_2-fixing and NO_3^--reducing organisms[17]. During the present investigation I examined in the same organism *(N. muscorum)* whether vanadium (V) can function in place of Mo to any extent for N_2 fixation and/or NO_3^- reduction. The results show that while V fails to replace Mo functionally, like W or Cr[15,16], it stimulates the normal growth of the

organism on N_2- and NO_3^--containing media when applied in traces with Mo, but is inhibitory to the alga at a higher concentration.

The filamentous, heterocystous, N_2-fixing and NO_3^--metabolizing blue-green alga *Nostoc muscorum*, obtained from the Department of Botany, Banaras Hindu University, Varanasi, India, has been used as the test material during the present investigation. This alga does not form heterocysts in 5 mM NO_3^- medium, owing to their inhibition by active nitrate reductase during NO_3^- metabolism[18]. When transferred from NO_3^- to nitrogen-free (N_2) medium the alga starts forming heterocysts with a frequency of 5–6% within 20–24 h.

Experimental cultures of the alga were grown routinely in modified Chu 10 medium[19] under the culture conditions described previously[20,21]. The NO_3^--growing (non-heterocystous) log-phase culture of the organism was harvested and washed with sterile N_2 medium (in order to remove traces of NO_3^-) and transferred to a fresh N_2 medium (in order to remove traces of NO_3^-) and transferred to a fresh N_2, 5 mM NO_3^- or 5 mM NO_2^- medium with or without 0.0177 ppm Mo, unsupplemented or supplemented with various concentrations of V. The samples (in triplicate) were incubated in the culture chamber and the amount of growth (by optical density determinations at 663 nm) and maximum heterocyst frequency (by microscopic examination in terms of number of heterocysts per hundred vegetative cells) were assessed on the 7th day of inoculation. The experiments were repeated 5 times and the results were analyzed statistically for biological significance and reproducibility.

Mo and V were used in the form of sodium molybdate and sodium vanadate (obtained from Sigma Chemical Co., USA) at the highest purity level. These chemicals were filter-sterilized and then added to the already sterile (autoclaved) mineral medium under aseptic conditions. All other chemicals (medium constituents) were obtained from British Drug House, Poole, Dorset, England.

The table illustrates the effects of varying V concentrations on *Nostoc muscorum* growing with or without Mo in N_2, NO_3^- or NO_2^- medium. The alga grew well in N_2, NO_3^- or NO_2^- medium containing 0.0177 ppm Mo. NO_2^- was apparently the best source of nitrogen for supporting growth of the alga, followed by NO_3^- and N_2. The organism formed heterocysts with a frequency of 5–6% in N_2 medium. There was a failure of algal growth in Mo-free N_2 and NO_3^- media coupled with the formation of heterocysts in NO_3^- medium. Such inhibition of growth and induction of heterocyst differentiation in the Mo-free cultures were not found at all in NO_2^- medium, as they are due to inhibition of the Mo-dependent nitrogenase and nitrate reductase, and nitrite reductase does not require Mo. Thus, the absence of Mo cuased a dysfunction of the nitrogenase and nitrate reductase enzyme systems, resulting in growth inhibition in N_2 and NO_3^- media. Since active nitrate reductase is known to repress the formation of heterocysts[18], its dysfunction relieved the repression, so that heterocysts were formed in NO_3^- medium.

Addition of V to Mo-free N_2- and NO_3^--grown cultures brought about an increase in their heterocyst frequency, therefore, suggesting that V has caused an acute nitrogen starvation in the Mo-free cultures in both N_2 and NO_3^- media. Enhancement of heterocyst frequency in response to acute nitrogen crisis has already been studied in *Nostoc muscorum*[15–17].

In Mo-containing medium, V was found to exert variable (dual) effects depending upon the concentrations applied. Up to a concentration of 0.0125 ppm, V supported a significantly better growth of the organism in N_2 and NO_3^- media, indicating that V could stimulate the activities of nitrogenase and nitrate reductase enzyme systems at its lower concentrations. V was found to inhibit N_2- and NO_3^--mediated growth of *Nostoc muscorum* significantly, and to induce the formation of heterocysts in NO_3^- medium. V could neither enhance or retard the growth of the alga in NO_2^- medium, nor induce the formation of heterocysts in NO_2^- medium. Thus, the inhibitory effects of higher concentrations of V appear to be exclusively operative at the level of the nitrogenase and nitrate reductase enzyme systems. This phenomenon can be explained as V replacing Mo on the basis of their close structural similarity[22,23], and therefore, inactivating the Fe-Mo- and Mo-containing co-factors of the nitrogenase and nitrate reductase enzyme systems, unlike W or Cr, which are known functionally to replace Mo for nitrogenase and nitrate reductase activities in *N. muscorum* strains[15,16]. However, the growth-promoting efficiency of the lower concentrations of V may not be due to its functioning in place of Mo. Had it been functioning like Mo at its lower concentrations, it would have supported growth of the alga in N_2 and NO_3^- media even in the absence of Mo. The effect of V, therefore, appears to be due to its involvement, at lower concentrations, in stabiliz-

Data* on growth** and heterocyst frequency*** of parent *Nostoc muscorum* in N_2, 5 mM NO_3^- or 5 mM NO_2^- medium (containing or lacking 0.0177 ppm sodium molybdate), unsupplemented or supplemented with various concentrations of sodium vanadate

±Mo	V (ppm)	Growth			Maximum heterocyst frequency	
		N_2	NO_3^-	NO_2^-	N_2	NO_3^-
−Mo	0.0000	0.0	0.0	0.415±0.012	6.8±0.6	6.5±0.3
−Mo	0.0100	0.0	0.0	0.415±0.014	7.1±0.5	6.5±0.8
−Mo	0.0125	0.0	0.0	0.416±0.017	7.1±0.2	6.8±0.3
−Mo	0.0150	0.0	0.0	0.415±0.015	7.4±0.5	6.9±0.4
−Mo	0.0175	0.0	0.0	0.420±0.012	7.6±0.4	6.9±0.1
−Mo	0.0200	0.0	0.0	0.410±0.015	7.6±0.3	6.8±0.4
+Mo	0.0000	0.325±0.015	0.370±0.014	0.416±0.013	5.5±0.3	0.0
+Mo	0.0100	0.330±0.012	0.385±0.016	0.415±0.012	5.5±0.4	0.0
+Mo	0.0125	0.375±0.014	0.405±0.015	0.415±0.014	5.6±0.8	0.0
+Mo	0.0150	0.320±0.013	0.365±0.014	0.417±0.015	6.2±0.3	3.2±0.3
+Mo	0.0175	0.255±0.014	0.305±0.012	0.416±0.014	6.4±0.2	4.8±0.5
+Mo	0.0200	0.160±0.012	0.215±0.013	0.415±0.015	6.8±0.4	5.4±0.2

*The values are means of 5 independent readings with their respective SE. **Similar relative quantitative effect(s) of Mo and/or V were observed from the day of inoculation of the alga to the advent of its exponential growth phase, i.e., on the 7th day (in proportion to the period of incubation), thus data on the final growth yield (on 7th day) has only been given (increase in OD at 663 nm). ***Number of heterocysts per hundred vegetative cells. Each individual reading is based on a random sampling of 12 algal filaments (maximum frequency was recorded on the 5th–7th day after inoculation). Heterocysts were never found in NO_2^- medium.

ing the Mo-protein binding in nitrogenase and nitrate reductase.

Earlier biochemical studies have clearly shown that V influences the rate of N_2 fixation in *Azotobacter* species by stabilizing Mo-protein binding in nitrogenase[24]. The present study, therefore, strongly indicates that V at a lower concentration has a role in stabilizing the Mo-protein binding of nitrogenase and nitrate reductase in *Nostoc muscorum*. Although there have been earlier reports on the occurrence of a V-nitrogenase[25] for N_2 fixation in *A. vinelandii* and *A. chrococcum*[26], and *Clostridium butyricum*[27], these unusual results need confirmation, because others are of the opinion that these organisms still contain traces of Mo which are responsible for nitrogenase activity[24]. The present results too emphasize that V is incapable of functionally replacing Mo, though it can help Mo-protein binding in nitrogenase for an increased N_2 fixation. Further in vitro studies on Mo-V interactions at the level of Fe-Mo- and Mo-containing co-factors of nitrogenase and nitrate reductase, as well as in vivo effects of graded V concentrations on these enzyme activities, may lead to the use of V as a trace element to increase N_2-fixing potential in this group of organisms.

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Inhibition of the ureteral contractions induced by rat urine with kallikrein antibodies[1]

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Summary. Purified urinary kallikrein induces contractions of the rat ureter in vitro. Antibodies against kallikrein block the contractile response of the isolated ureter to rat urine.

We have previously shown that rat urine induces contractions of the isolated rat ureter[2]. This effect was attributed to urinary kallikrein, since it was abolished by the serine protease inhibitor aprotinin and also by acid and heat treatment of the urine, but not by dialysis. To test the validity of this assumption, we investigated the effect of purified kallikrein on the muscular tone of the isolated ureter, as well as the capacity of kallikrein antiserum to block the urine - induced ureteral contractions.

Methods. Wistar rats (200-300 g b.wt) were placed in stainless steel metabolic cages, fed a normal rat chow and given free access to drinking water. Urine was collected at room temperature in plastic containers for 24-h periods and stored frozen. To purify urinary kallikrein, the urine was concentrated by ultrafiltration (100:1) and dialysed against 6.7 mM citrate/10 mM phosphate/11.4 mM borate buffer pH 9.5[3]. The concentrate was applied to a chromatography column (1 × 10 cm) containing aprotinin bound to CNBrSepharose[4]. Kallikrein elution, which was achieved with a pH gradient occurred between pH 3.6 and 4.5. The fractions containing the enzyme were pooled and applied to a Sephadex G-100 column (5 × 100 cm) which was developed with phosphate buffer (0.1 M, pH 5.5). The fractions which contained the kallikrein activity were pooled, concentrated by ultrafiltration, dialysed against distilled water and freezedried. Purified kallikrein (15 µg/cm^2) was found to run as a single band on SDS electrophoresis.

Kallikrein (100 µg) emulsified in complete Freund adjuvant was injected intradermally (i.d.) in multiple sites of the back of a New Zealand rabbit. The injections were applied once a week for 5 weeks. A month later the same amount of kallikrein emulsified in imcomplete Freund adjuvant was injected i.d. Blood was drawn before immunization and 1 week after after each injection. The antiserum was heated at 56 °C for 30 min and stored frozen[4].